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Defects in homocysteine metabolism: Diversity among hyperhomocyst(e)inemias

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Abstract

There are now four genetic mouse models that induce hyperhomocyst(e)inemia by decreasing the activity of an enzyme involved in homocysteine metabolism: cystathionine β -synthase, methylenetetrahydrofolate reductase, methionine synthase and methionine synthase reductase. While each enzyme deficiency leads to murine hyperhomocyst(e)inemia, the accompanying metabolic profiles are significantly and often unexpectedly, different. Deficiencies in cystathionine β -synthase lead to elevated plasma methionine, while deficiencies of the remaining three enzymes lead to hypomethioninemia. The liver [S-adenosylmethionine]:[S-adenosylhomocysteine] ratio is decreased in mice lacking methylenetetrahydrofolate reductase or cystathionine β -synthase, but unexpectedly increased in mice with deficiencies in methionine synthase or methionine synthase reductase. Folate pool imbalances are seen in complete methylenetetrahydrofolate reductase deficiency, where methyltetrahydrofolate is a minor component, and in methionine synthase reductase deficiency, where methyltetrahydrofolate is increased relative to wild-type mice. These differences illustrate the potential diversity among human patients with hyperhomocyst(e)inemia, and strengthen the argument that the pathologies associated with the dissimilar forms of the condition will require different treatments.

Keywords

adenosylhomocysteine; adenosylmethionine; cystathionine β -synthase; folate; genetic mouse models; homocysteine; methionine synthase; methionine synthase reductase; methylenetetrahydrofolate reductase

Introduction

The literature is replete with extensive studies of hyperhomocyst(e)inemia in humans. Hyperhomocyst(e)inemia may arise from a combination of dietary and/or genetic factors, including polymorphisms or severe mutations in enzymes involved in homocysteine metabolism and dietary deficiencies of vitamin B6, riboflavin, cobalamin and/or folate. The enzymes whose deficiency gives rise to hyperhomocyst(e)inemia include cystathionine β -synthase, methylenetetrahydrofolate reductase, methionine synthase and methionine synthase reductase. Despite the common factor of an elevation in plasma homocysteine, patients with these enzyme deficiencies may display rather varied adverse clinical effects. Deficiencies of methylenetetrahydrofolate reductase and methionine synthase give rise to low or normal levels of plasma methionine (1), while deficiencies of cystathionine β -

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synthase lead to hypermethioninemia (2). The pathologies associated with mutations leading to severe enzyme deficiencies have been attributed to defects in cellular methylation reactions due to altered ratios of [*S*-adenosylmethionine (AdoMet)]:[*S*-adenosylhomocysteine (AdoHcy)], to oxidative stress and covalent modifications of proteins induced directly by elevated plasma homocysteine and indirectly by homocysteine-thiolactone, or in the case of methionine synthase deficiency to methyl trapping leading to an imbalance in folate pools. If increased cardiovascular risk were due to a direct effect of elevated plasma homocyst(e)ine, one might expect that protocols that lower plasma homocyst(e)ine such as folate supplementation would ameliorate the risk. In the face of disappointing results of folate fortification during secondary prevention trials (HOPE-2 (3), VISP (4), and NORVIT (5)) in reducing cardiovascular events in patients with a prior history of cardiovascular disease, we are forced to ask whether the increased cardiovascular disease risk associated with hyperhomocyst(e)inemia might not have more complex causes, depending on the associated metabolic abnormalities. For example, methyl trapping, or the accumulation of methyltetrahydrofolate at the expense of 10-formyltetrahydrofolate and methylenetetrahydrofolate needed for the biosynthesis of the nucleotide precursors of RNA and DNA, is expected to be a problem in hyperhomocyst(e)inemia caused by deficiencies of methionine synthase or methionine synthase reductase, but not in deficiencies of methylenetetrahydrofolate reductase, where the level of methyltetrahydrofolate is abnormally low. However, measurements of folate distributions in the tissues of humans that would confirm this hypothesis are lacking. We will begin with a description of how mouse models of hyperhomocyst(e)inemia as a group serve to illustrate the possible complexities associated with the human forms of the condition. We will then discuss relevant human clinical findings in light of these findings in genetic mouse models.

Metabolite measurements in genetic mouse models for hyperhomocyst(e)inemia

Genetic mouse models of hyperhomocyst(e)inemia are extremely relevant to our understanding of hyperhomocyst(e)inemia in humans because they permit tissue metabolite measurements that are difficult if not impossible to obtain in affected humans. To date there are four strains of mice in which hyperhomocyst(e)inemia has been induced by genetic manipulations leading to a deficiency of one of the enzymes involved in homocysteine metabolism.

Cystathionine β -synthase.

The first genetic model for hyperhomocyst(e)inemia lacked the *Cbs* gene specifying cystathionine β -synthase. Cystathionine β -synthase catalyzes the first step in the conversion of homocysteine to cysteine: the condensation of homocysteine and serine to form cystathionine. As expected, mice with this enzyme deficiency have elevated plasma homocyst(e)ine (6). *Cbs* heterozygotes show mildly elevated plasma methionine and normal levels of AdoMet, but show elevations in liver AdoHcy resulting in a reduced [AdoMet]:[AdoHcy] ratio in this tissue (7). In the severely hyperhomocyst(e)inemic *Cbs* mutant homozygote, AdoMet levels in liver are *elevated* approximately 2-fold, while AdoHcy levels are elevated about 9-fold, again resulting in a reduced AdoMet:AdoHcy ratio and impaired genomic DNA methylation status (8). These authors reported that the effect of the *Cbs* knockout on AdoMet levels was tissue specific, with almost no effect in brain or kidney.

Methylenetetrahydrofolate reductase.

A knockout of the *Mthfr* gene specifying methylenetetrahydrofolate reductase was constructed in the Rozen laboratory (9). *Mthfr*^{-/-} mice have elevated plasma homocyst(e)ine and reduced plasma methionine. Their AdoMet levels were lower in all tissues examined,

and AdoHcy levels were higher, resulting in AdoMet:AdoHcy ratios that were dramatically decreased (9). Biological methylation was impaired in all tissues examined. These mice also exhibited an abnormal distribution of folates: methyltetrahydrofolate levels are greatly reduced and the total folate content was unchanged in liver and brain (9) but reduced in plasma (10). While methyltetrahydrofolate is the major folate in the plasma of wild-type animals, it comprises less than 40% of the folate in *Mthfr*^{-/-} mice.

Methionine synthase.

A knockout of the *Mtr* gene specifying cobalamin-dependent methionine synthase results in embryonic lethality (11). Heterozygotes show mildly elevated plasma homocyst(e)ine and normal (12) or *elevated* (11) plasma methionine. In liver, AdoMet levels were slightly increased in heterozygotes as compared to wild-type mice, while AdoHcy levels were slightly decreased, although the changes did not reach statistical significance.

Methionine synthase reductase.

A knockout of the *Mtrr* gene specifying methionine synthase reductase, an auxiliary protein required for methionine synthase activity, also causes embryonic lethality in homozygotes (unpublished data of Roy Gravel, discussed in (13)). A gene-trap insertion (*Mtrr*^{Gt}(pGT1Lxf)XG334Byg; hereafter abbreviated *Mtrr*^{Gt}) into this gene results in an *Mtrr*^{Gt/Gt} mouse methionine synthase reductase activity that is reduced but not absent (13). The *Mtrr*^{Gt/Gt} mouse is hyperhomocyst(e)inemic and hypomethioninemic. However, the liver levels of AdoMet are *elevated* and the levels of AdoHcy are *decreased*. This surprising observation suggests unidentified compensatory mechanisms exist in the liver that can spare AdoMet in a hypomethioninemic mouse. [AdoMet]:[AdoHcy] ratios in brain and heart are unchanged by *Mtrr* disruption. The *Mtrr*^{Gt/Gt} mouse shows a tendency towards methyl trapping, with elevated fractions of methyltetrahydrofolate in liver, heart and kidney, but not in brain. The metabolite measurements in these various mouse models are qualitatively summarized in Table 1.

What do these models tell us that is of relevance to hyperhomocyst(e)inemia in humans?

Folate metabolism.

None of the mouse models that have been examined show altered levels of total folates in tissues. It will be of great interest to see if their hyperhomocyst(e)inemia responds to folate supplementation of standard lab chow. A preliminary indication of the response of *Mthfr*-deficient mice to folate deficiency comes from studies in the Rozen laboratory (14). A folate-deficient diet exacerbates hyperhomocyst(e)inemia in both wild-type and *Mthfr*^{+/-} mice, suggesting that addition of folate to a normal diet might reduce plasma homocyst(e)ine levels in *Mthfr*-deficient mice, as it is known to do in humans with mild MTHFR deficiency due to homozygosity for the 677C>T mutation (15). However, it remains to be determined whether folate supplementation would reduce plasma homocyst(e)ine in mice with deficiencies in methionine synthase or methionine synthase reductase induced by genetic manipulation or by cobalamin deficiency, or in *Cbs*-deficient mice. The most common genetic cause of human hyperhomocyst(e)inemia is the homozygosity for the 677C>T mutation in MTHFR (15), and we know that the plasma homocyst(e)ine of TT patients is responsive to dietary folate supplementation. However, other causes of hyperhomocyst(e)inemia might not respond to folate supplementation, or might respond without altering the root cause of cardiovascular risk.

Embryonic lethality.

Of the four genetic mouse models for hyperhomocyst(e)inemia discussed here, the only enzyme deficiencies that lead to embryonic lethality are deficiencies in methionine synthase and methionine synthase reductase. Thus clearly, hyperhomocyst(e)inemia in and of itself is not sufficient to cause embryonic lethality. These are the two enzyme deficiencies most likely to lead to methyl trapping, and it is tempting to speculate that intracellular methyl trapping is linked to the embryonic lethality. If so, conditions which lead to milder methyl trapping in humans and in mice (e.g. cobalamin deficiency, or polymorphism in methionine synthase or methionine synthase reductase) may also be associated with increased incidence of fetal loss. Unpublished studies on pregnancy outcome in the *Mtrr*^{+/Gt} dam in the Rozen laboratory suggest that maternal methionine synthase reductase deficiency is indeed linked to embryonic resorption and fetal abnormalities, including ventricular septal defects. The implications of these results are profound, in light of the recent meta-analysis that indicated maternal low folate status is a risk factor for spontaneous abortion (16), since the critical form(s) of folate are not known.

Neural tube defects.

Finally there is the issue of the linkage between hyperhomocyst(e)inemia and neural tube defects. No studies that we are aware of have reported the incidence of neural tube defects in hyperhomocyst(e)inemic mice. Cardiac abnormalities, particularly ventricular septal defects, are often used as surrogate markers for the much rarer neural tube defects, and ventricular septal defect incidence has been shown to be elevated in wild-type mice fed a folate-deficient diet, and in both *Mthfr*^{+/-} and *Mtrr*^{Gt/Gt} mice (13, 14). But more comprehensive studies might help to elucidate the origin of the dramatic effect of maternal periconceptual folate supplementation in the periconceptual period in lowering the incidence of neural tube defects in the resultant offspring.

Conclusions

Given the very different metabolite profiles observed in genetic mouse models for hyperhomocyst(e)inemia, we need to emphasize the idea that some or all of the pathological risks associated with human hyperhomocyst(e)inemia may arise not from an elevation in homocyst(e)ine per se, but from metabolic abnormalities that are specific to the cause of that hyperhomocyst(e)inemia.

Abbreviations:

Cbs	Cystathionine β -synthase gene
Mtr	methionine synthase gene
Mtrr	methionine synthase reductase gene
Mthfr	Methylenetetrahydrofolate reductase gene
AdoHcy	S-adenosylhomocysteine
AdoMet	S-adenosylmethionine

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Table 1

genetic mouse models for hyperhomocyst(e)inemia^a

plasma Hcy	plasma Met	liver AdoMet	liver AdoHcy	liver ratio AdoMet:AdoHcy	liver total folate	liver % CH ₃ - H ₄ folate	References
<i>Clin Chem Lab Med.</i> Author manuscript; available in PMC 2011 May 20.				not determined	not determined	Choumenovitch et al., 2002

	plasma	plasma	liver	liver	liver	liver	liver	liver	References
	Hcy	Met	AdoMet	AdoHcy	ratio AdoMet:AdoHcy	total folate	% CH ₃ - H ₄ folate		Dayal et al, 2001
	unchanged			not determined	not determined		

	plasma	plasma Met	liver AdoMet	liver AdoHcy	liver ratio AdoMet:AdoHcy	liver total folate	liver % CH ₃ - H ₄ folate	References
	not determined				unchanged		Chen et al., 2001

	plasma Hcy	plasma Met	liver AdoMet	liver AdoHcy	liver ratio AdoMet:AdoHcy	liver total folate	liver % CH ₃ - H ₄ folate	References
	not determined				unchanged		Chen et al, 2001
	unchanged	not determined	Swanson et al, 2001; Dayal et al., 2005

plasma Hcy	plasma Met	liver AdoMet	liver AdoHcy	liver ratio AdoMet:AdoHcy	liver total folate	liver % CH ₃ - H ₄ folate	References
unchanged		Elmore et al., 2007
not determined	not determined	not determined	not determined	not determined	Elmore et al., 2007

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Site levels are relative to the wild-type littermates. Unexpected changes are highlighted in red.